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EDITORIAL COMMENTARY

# Approaches to quantify radioligands that wash out slowly from target organs

**Jussi Hirvonen · Garth E. Terry · Christer Halldin · Victor W. Pike · Robert B. Innis**

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Sanabria-Bohórquez and colleagues [1] reported methods to quantify the uptake in human brain of radioligand for cannabinoid $CB_1$ receptors, $^{18}$F-MK-9740, that washes out very slowly from this organ. The slow washout is caused, in part, by the high affinity of the radioligand, but binding is presumably not irreversible in humans, since it can be rapidly displaced from monkey brain [2]. Furthermore, modest washout from human brain can be observed but only by scanning for almost 12 h. Due to the slow washout and a more limited period of data for quantitation (typically 2–6 h), models of reversible binding for $^{18}$F-MK-9740 did not appropriately fit the data. To overcome this problem, Sanabria-Bohórquez and colleagues [1] simplified the reversible model by assuming a similar off-rate from receptors ($k_4$) across brain regions and then validated several models of irreversible binding against this modified model of reversible binding[1]. We think this approach to analyze a radioligand with slow washout from brain is flawed because $k_4$ is not uniform across brain regions and because an irreversible model violates the known reversible property of the radioligand in monkey brain. The purpose of this commentary is to suggest approaches to analyze radioligands with slow washout from brain. Our suggestions are based, in part, on our own experience with other $CB_1$ receptor radioligands that also have slow washout from brain.

We also struggled to quantify slow brain washout using two radioligands for the $CB_1$ receptor labeled with either $^{11}$C or $^{18}$F: $^{11}$C-MePPEP [4] and $^{18}$F-FMPEP-$d_2$ [3]. Slow washout from brain can be caused by slow peripheral clearance, high affinity of the radioligand, and/or high density of receptors. In fact, slow washout of our radioligands was due to all three properties, and, as will be discussed below, slow plasma clearance was particularly problematic. One basic problem that both Sanabria-Bohórquez and colleagues and we had was to identify the "gold standard" against which to measure bias and accuracy of various methods of quantitation. Of course, we rarely have the opportunity to compare in vivo results with postmortem or surgical samples in human subjects. Fur-

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J. Hirvonen · G. E. Terry · V. W. Pike · R. B. Innis (✉)
Molecular Imaging Branch, National Institute of Mental Health,
National Institutes of Health,
31 Center Drive, MSC-2035,
Bethesda, MD 20892-2035, USA
e-mail: robert.innis@nih.gov

C. Halldin
Department of Clinical Neuroscience, Psychiatry Section,
Karolinska Institutet,
Stockholm, Sweden

[1] Sanabria-Bohórquez and colleagues [1] recommended a method they call "fractional uptake ratio" (FUR) to analyze brain and plasma data from $^{18}$F-MK-9470. FUR equals the ratio of the concentration of radioactivity in the brain at the end of the scan to the area under the plasma time-activity curve from time zero to the end of the scan. The rationale for this choice was based on a three-part argument. First, $V_T$ using a fixed value for $k_4$ was assumed to be accurate. Second, $K_i$, the irreversible uptake constant, correlated with $V_T$ and was, therefore, concluded to be accurate. Third, FUR correlated with $K_i$ and was similarly concluded to be accurate. Please also note that FUR is not a model-based outcome measure and that it could be applied to both reversibly and irreversibly binding radioligands. Because FUR measures only the last time point in brain, it is vulnerable to variability in washout rates in brain. Furthermore, because plasma is not extrapolated to infinity, FUR is vulnerable to variability in terminal clearance of the radioligand. For example, the area under the plasma curve of $^{18}$F-FMPEP-$d_2$ within the first 2 h of injection is only about 50% of the total area under the curve extrapolated to infinity [3]. However, this commentary will not focus on the strengths and limitations of the method they call FUR. Instead, it will critique the first two of the three-part argument, i.e., validity of fixing $k_4$ and the use of irreversible models.

thermore, such in vitro measurements may not be the true gold standard because binding conditions vary in vitro and in vivo or because of rapid postmortem changes in the phosphorylation status of the receptor [5]. Our field does not have universally accepted criteria to select the "gold standard," but we suggest that the "best" model is the one that incorporates the known biological and pharmacological properties of the radioligand and its target. For example, if the radioligand is known to be reversibly bound, then a reversible model is a priori more accurate than an irreversible model. In this regard, we think that Sanabria-Bohórquez and colleagues have mistakenly recommended an irreversible model to analyze brain uptake of $^{18}$F-MK-9740. If such a model is recommended for practical purposes (e.g., limited data acquisitions), then the limitations of the model should be carefully assessed relative to a gold standard. However, Sanabria-Bohórquez and colleagues used a "gold standard" by assuming $k_4$ is uniform across brain regions, which is known to be invalid.

Early models for reversible radioligands assumed that in vivo $k_4$ was equal to the in vitro dissociation rate constant ($k_{off}$) [6]. Since $k_{off}$ is a property of the receptor for a particular radioligand, then both $k_{off}$ and $k_4$ should be uniform across brain regions if they express the same receptor. However, subsequent studies showed that the in vivo $k_4$ is often many fold slower than in vitro $k_{off}$. For example, the in vivo $k_4$ of [$^{123}$I]iomazenil (a probe for the $\gamma$-aminobutyric $acid_A$ receptor) is 20-fold slower than its in vitro $k_{off}$, even using in vitro conditions that try to mimic those in vivo [7]. This difference between $k_4$ and $k_{off}$ is thought to be caused by re-binding of the radioligand [8]. That is, in vivo $k_4$ is often slower than in vitro $k_4$, because the efflux of the radioligand from the specific compartment is delayed by repeated re-binding to receptors in the immediate area. For example, this discrepancy is clear from the unusually slow $k_4$ (0.01 $min^{-1}$) of $^{11}$C-MePPEP which would not permit such a rapid displacement of radioligand from monkey brain (>50% in 20 min) [9]. Pharmacological doses of the displacing agent block not only the radioligand but also virtually all available receptors. Blocking these receptors prevents re-binding and thereby drives the radioligand from the specific compartment, making it available for removal via veins. This phenomenon of re-binding has been called the "synaptic barrier" [10] and is thought to correlate with the local density of receptors. Thus, as a general rule, the washout of radioligand is slower from those regions with higher receptor densities.

How should investigators address the problem of quantifying a radioligand with slow kinetics? We recommend three general approaches. First, studies in animals often clarify important pharmacological properties of the radioligand. In this case, scanning in monkeys has shown that all three $CB_1$ radioligands ($^{18}$F-MK-9740, $^{11}$C-MePPEP, and $^{18}$F-FMPEP-$d_2$) bind reversibly, because they can be displaced by pharmacological doses of nonradioactive ligands [2, 3, 9]. Thus, the model for quantitation should either incorporate these pharmacological properties or carefully examine limitations of violating these properties. Second, long scans are typically required to identify rate constants of radioligands with slow kinetics. In our case and contrary to common perception in the field, we showed that $^{11}$C-MePPEP can be reliably quantified for 210 min after injection, that is, for more than ten half-lives of the radionuclide $^{11}$C [4]. Third, we found that retest studies can be unusually useful to identify sources of bias and variability in quantitation of the radioligand. A typical outcome measure for radioligands is distribution volume ($V_T$), which is the ratio at equilibrium of the concentration of radioligand in brain to that in plasma. Viewed as a fraction, errors in $V_T$ can derive from either the numerator (concentration of radioligand in brain) and/or the denominator (concentration of radioligand in plasma). We performed a retest study in healthy subjects of both $^{11}$C-MePPEP [4] and $^{18}$F-FMPEP-$d_2$ [3]. As a surrogate for the equilibrium concentration in brain, we measured brain uptake at the time of relative stability. As a surrogate for the equilibrium concentration of radioligand in plasma, we calculated the area under the curve from time zero to infinity. We expected that $^{18}$F-FMPEP-$d_2$ would be more accurate than $^{11}$C-MePPEP largely because the longer half-life of $^{18}$F would allow longer scanning and better identifiability of the rate of washout from brain. To our surprise, our studies strongly suggested that the major source of error in estimation of $V_T$ derived from measurements in plasma rather than those in brain. That is, the terminal clearance of both radioligands is very slow, and the longer half-life of $^{18}$F-FMPEP-$d_2$ compared to that of $^{11}$C-MePPEP allowed later plasma samples to be measured and, thereby, to better identify the terminal clearance of the radioligand. In brief, the denominator component (plasma) of $V_T$ for $^{11}$C-MePPEP was likely a greater source of error than the numerator (brain) component. For $^{18}$F-MK-9740, Sanabria-Bohórquez and colleagues measured the reproducibility of only brain uptake, which is typical for an irreversible radioligand. Thus, we do not know the impact of errors in plasma measurements on the accuracy of $^{18}$F-MK-9740 in human subjects.

In summary, although our field does not have universally accepted criteria to select the "gold standard" against which to measure simplified approaches, we suggest that the "best" model is the one that incorporates the known pharmacological properties of the radioligand and its target. Sanabria-Bohórquez and colleagues did not follow this suggestion. Instead, they assessed irreversible models (which is contrary to the known pharmacology of the radioligand) and tried to validate the approach using a reversible model with an assumption that is likely erroneous (i.e., $k_4$ is uniform across brain regions). As a general approach to radioligands with slow kinetics, we recommend that studies in animals be used to determine key

pharmacological properties, like reversibility, of the radioligand; that long scans (and sometimes atypically long scans) be used to identify slow rate constants; and that retest studies can be unusually useful to identify sources of error in measurements of brain uptake and the concentration of radioligand in plasma, as well as in distribution volume ($V_T$), which is a ratio of brain to plasma measurements.

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**Conflicts of interest** None.

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